Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Tunnel Dielectrophoresis for Ultra-High Precision Size-based Cell Separation

Yu-Chun Kung, Kayvan R. Niazi, and Pei-Yu Chiou

Supporting Information

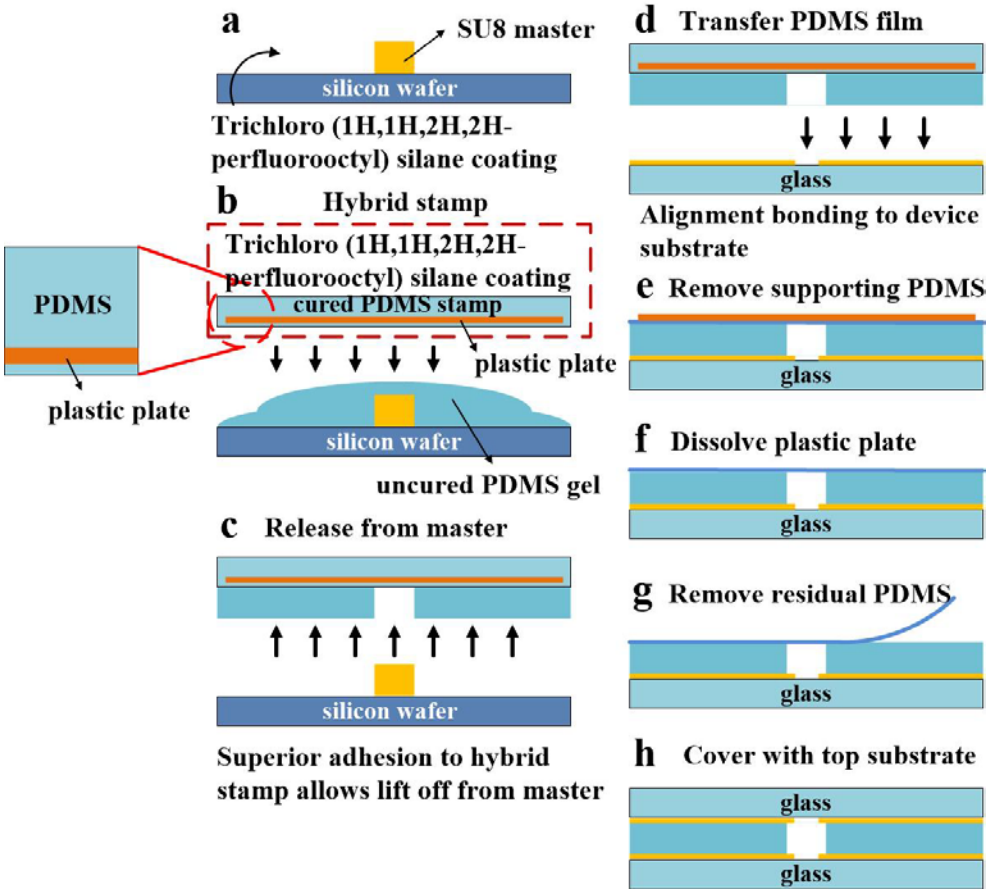


Figure S1 : Process flow for fabricating a TDEP device. (a) It starts from fabricating a SU8 mold (SU8 3050) on a silicon wafer by standard photolithography. The mold surface was treated with trichloro (1H,1H,2H,2H-perfluorooctyl) silane (97%, Sigma-Aldrich, USA), also called PFOCTS, to facilitate later demolding. This surface treatment was carried out in a vacuum chamber at a pressure of -30 psi for 16 hours. (b) A plastic embedded hybrid PDMS stamp was fabricated using the process mentioned previously by our group. [31] The hybrid stamp was also surface treated with PFOCTS for 6 hours. To fabricate a PDMS thin film with through-layer structures, uncured PDMS was poured onto the SU8 mold, pressed by the hybrid stamp at a

pressure of 4psi, and cured at 50^0C in an oven for an hour. (c) The PDMS film was demolded from the SU8 mold. The cured PDMS film has stronger adhesion to the hybrid stamp than the SU8 mold due to longer PFOCTS treatment. (d) The PDMS film was aligned, transferred, and bonded to a glass substrate with Au electrodes by oxygen plasma treatment. The bonded set was baked in an oven at 60^0C for 2 hours. In this study, the Au stripe electrodes were 100nm-thick on top of 10nm-thick Titanium. The metals were deposited by e-beam evaporation. Lift-off process was used to pattern of the electrodes. (e) The support PDMS was peeled off from the hybrid stamp. (f) The polystyrene plastic plate was dissolved in acetone. (g) A thin residual PDMS film on the substrate was peeled off from the device. (h) A top glass substrate with Au electrodes was aligned and bonded with the PDMS film by oxygen plasma treatment to complete the device fabrication process.

12

13

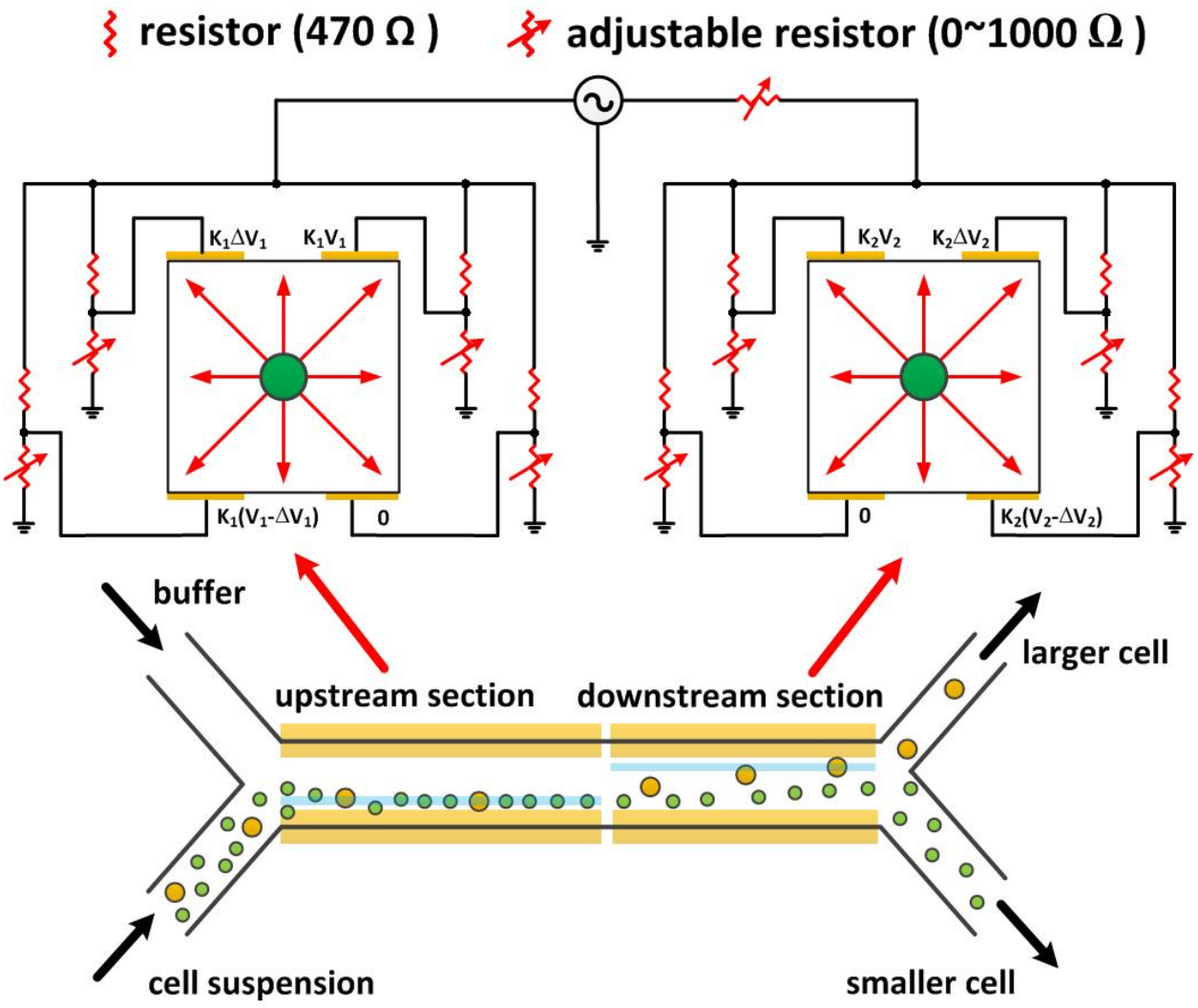


Figure S2 : Modulation circuits used to supply all different voltage combinations on FM and AM electrodes by sharing the single augmented signal.

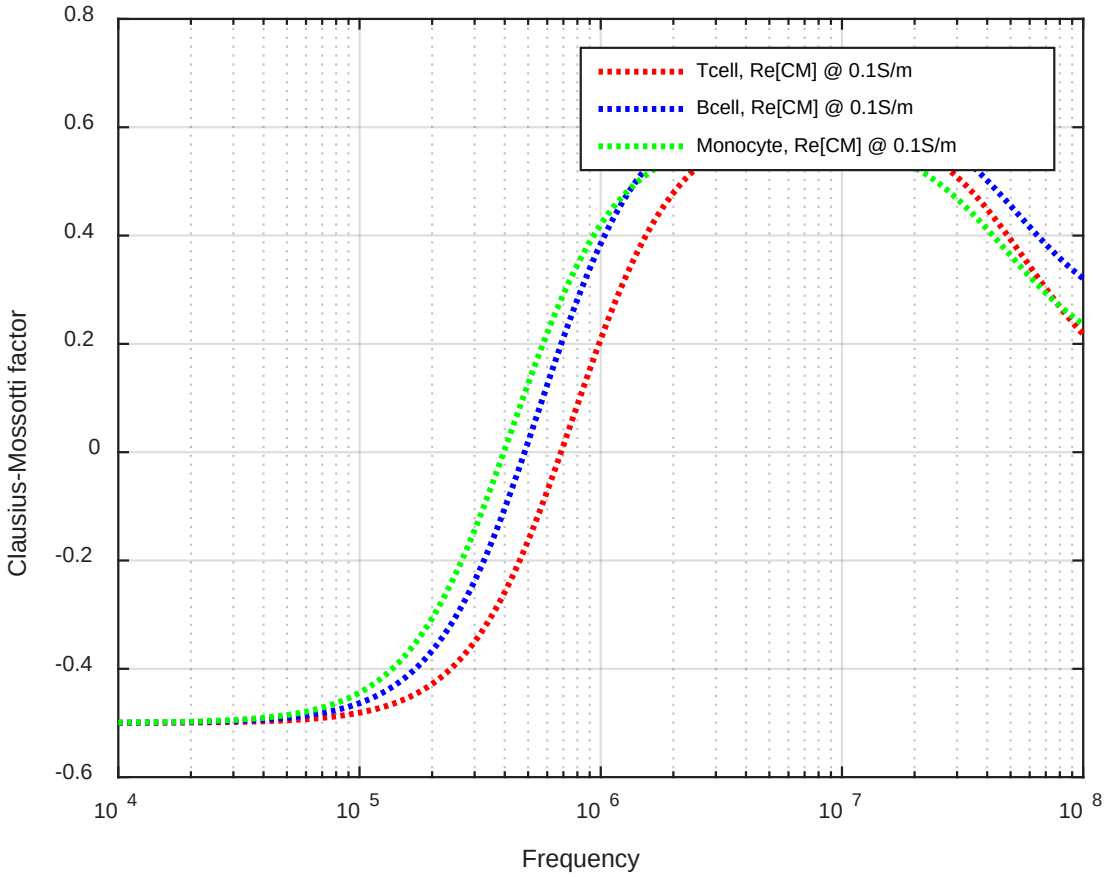


Figure S3 : The real part of the Clausius-Mossotti factor versus excitation frequency. The corresponding physical properties used to construct the *CM* curve are listed as follows, T lymphocyte (radius r = 3.6±0.55μm, cytoplasm relative permittivity ε_{cyto} = 100, cytoplasm conductivity σ_{cyto} = 0.53±0.1S·m^{-1}, specific membrane capacitance C_{mem} = 7.01±0.91mF·m^{-2}), B lymphocyte (radius r = 3.6±0.6μm, cytoplasm relative permittivity ε_{cyto} = 100, cytoplasm conductivity σ_{cyto} = 0.41±0.1S·m^{-1}, specific membrane capacitance C_{mem} = 10.33±1.6mF·m^{-2}), and monocyte (radius r = 4.8±0.55μm, cytoplasm relative permittivity ε_{cyto} = 100, cytoplasm conductivity σ_{cyto} = 0.37±0.15S·m^{-1}, specific membrane capacitance C_{mem} = 11.77±2.12mF·m^{-2}). The conductivity of the suspension medium (buffer) is 0.1S·m^{-1}.

Cell Size determination

The following are the steps of obtaining the size data of the cells in this study.

Step 1:

All the cell types used in this study were suspension type of cell, which have morphology mostly in spherical shapes. Thus, we assume each individual cell was approximated as a sphere with its corresponding diameter.

Step2:

With the microscope images taken under the same magnification, get the dimension scale on how many microns each pixel represents. In this study, each pixel is equivalent to 0.32μm. As an example, the original image can be referred to the following image. The cell in the enclosed red rectangle will be used to calculate its corresponding diameter in the later steps.

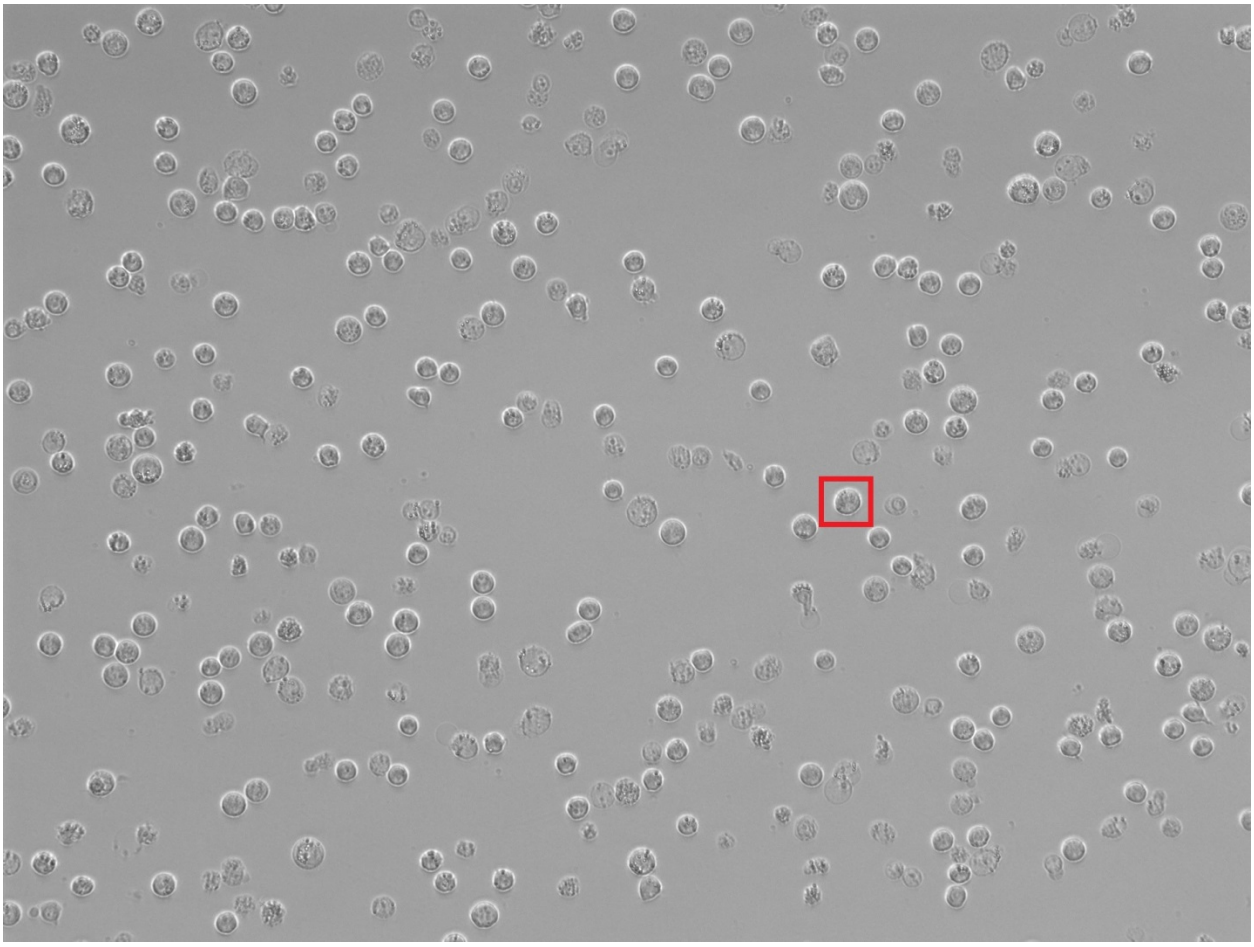
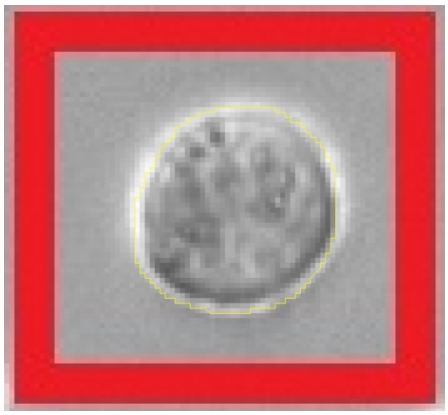


Figure S4-1 : Enclose the cell of interest for size analysis in the original microscope image.

Step3 :

Zoon-in the target cell, and manually enclose its boundary, then measure the enclosed area A_{cell}.



	Area	Mean	Min	Max
1	1332	159.351	90	251

Figure S4-2 : Zoom-in, enclose boundaries manually with closed polygon. Then calculate the enclosed area.

Step4 :

The diameter of the cell will be calculated as :

$$D_{cell} = 2 \times \sqrt{\frac{A_{cell}}{\pi}} \times 0.32$$

Step5 :

Repeat Step2 to Step4 for all the cells to get the size distribution.

Explanation of particle size purity measurement

In Table 1, the purities were measured by flow cytometer. Only the 10μm particles are fluorescent polystyrene beads. The 9μm, 12μm, and 15μm polystyrene beads are not fluorescent beads. As shown in Table 1, the three sets of particle mixtures were used, (9μm+10μm), (10μm+12μm), and (10μm+15μm). Thus, we could clearly identify the pre- and post-sorting purity based on the fluorescence count. In each set of mixture experiment, over 300 total number of particles were analyzed. The size distributions of the 9μm, 10μm, 12μm, and 15μm particles were 9±0.4μm, 10±0.05μm, 12±0.4~0.5μm, and 14.6±0.5μm, respectively. From which, the size variation the four different particle size categories were small. Also they were all made out of polystyrene material.

The example of the flow cytometer data for each set of experiments were shown here,

(9μm+10μm) :

- Initial mixture

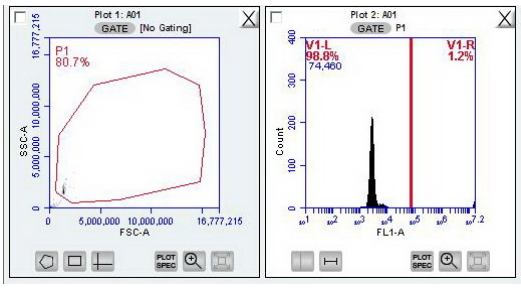


Figure S5-1 : Pre-sort purity of 9µm non-fluorescent and10µm green fluorescent polystyrene particles.

- After sorting

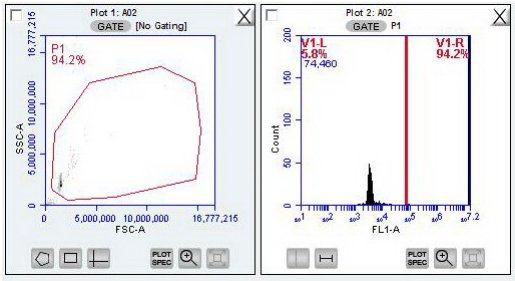


Figure S5-2 : After-sort purity from collection channel of 9µm non-fluorescent and 10µm green fluorescent polystyrene particles.

(<u>10µm+12µm</u>) :

- Initial mixture

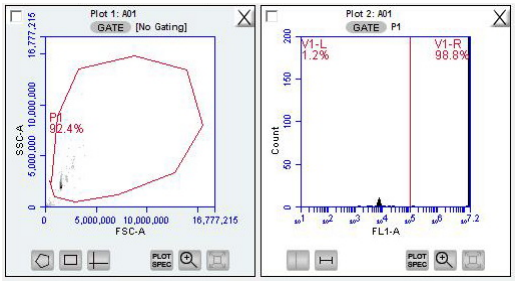


Figure S5-3 : Pre-sort purity of 10µm green fluorescent and 12µm non-fluorescent polystyrene particles.

- After sorting

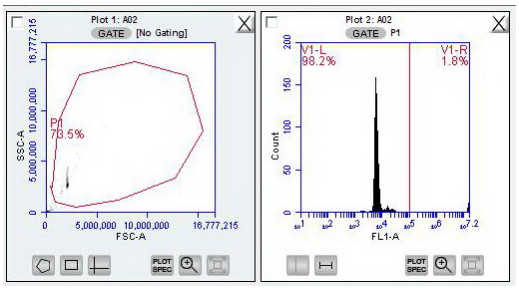


Figure S5-4 : After-sort purity from collection channel of 10µm green fluorescent and 12µm non-fluorescent polystyrene particles.

(<u>10µm+15µm</u>) :

- Initial mixture

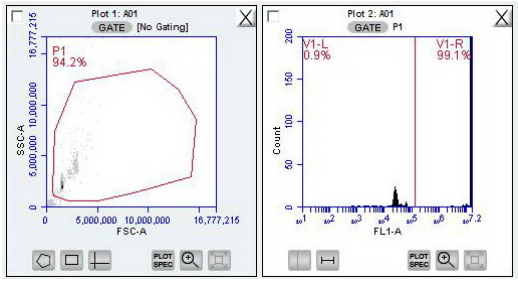


Figure S5-5 : Pre-sort purity of 10µm green fluorescent and 15µm non-fluorescent polystyrene particles.

- After sorting

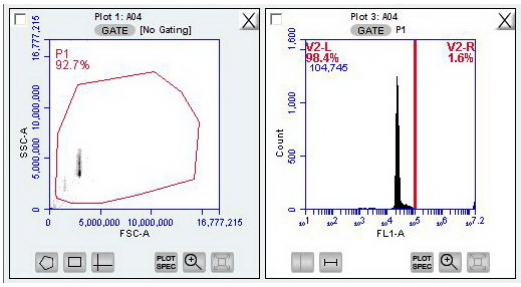


Figure S5-6 : After-sort purity from collection channel of 10µm green fluorescent and 15µm non-fluorescent polystyrene particles.

Video S4 : Movie of simulated particle trajectory of 10µm, 12µm, and 15µm of polystyrene particles during TDEP process.

Video S5 : Movie of TDEP upstream focusing and downstream separation of 10µm and 15µm polystyrene particles.

Video S6 : Movie of TDEP upstream focusing and downstream separation of 10µm and 12µm polystyrene particles.

Video S7 : Movie of TDEP upstream focusing and downstream separation of 9µm and 10µm polystyrene particles.

Video S8 : Movie of TDEP upstream focusing and downstream size separation of HL60 cells.

Video S9 : Movie of TDEP downstream size separation of PBMCs.